REMARKS

I. Status Summary

Claims 1-34 were filed with the subject application. Claims 1-10, 18-23, 25-29 and 34-38 are currently pending and have been examined by the U.S. Patent and Trademark Office (hereinafter "the Patent Office"). Claims 1-10, 18-23, 25-29 and 34-38 presently stand rejected.

Claims 18-23 and 37 have been rejected under 35 U.S.C. § 112, second paragraph, upon the Patent Office contention that the claims are indefinite for failure to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

Claims 1-4, 6-10, 18-23, 25-29 and 36-38 presently stand rejected under the provisions of 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 5,874,471 to Waugh (hereinafter referred to as "Waugh").

Claims 5 and 34-35 presently stand rejected under the provisions of 35 U.S.C. § 103(a) as allegedly being unpatentable over Waugh in view of U.S. Patent No. 5,767,160 to Kaesemeyer (hereinafter referred to as "Kaesemeyer").

Claims 1-4, 7-10, 18-23, 25-29 and 37-38 presently stand rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-5, 8-14 and 17-21 of U.S. Patent Application Serial No. 12/122,117 (hereinafter "the '117 application").

Claims 3, 7, 18, 20, 25, 27 and 37 have been canceled. Claims 1, 4, 8, 9, 19, 21-23, 26, 28, 29, 35, 36 and 38 have been amended to more particularly recite the presently disclosed subject matter. Support for the amendments can be found throughout the specification as filed, including particularly at page 46, lines 13-14; page 49, lines 22-24; page 73, lines 23-31; page 74, lines 10-23; page 76, line 21, through page 77, line 23; page 79, line 6-15; page 91, lines 5-9 and 23-26; in Table 4; throughout the Examples; and in claims 3, 7, 20 and 27 as originally filed. No new matter has been added.

New claims 39-42 have been added. Support for new claims 39-42 can be found throughout the specification as filed, including particularly at page 46, lines 13-14; page 49, lines 22-24; page 73, lines 23-31; page 74, lines 10-23; page 76, line 21, through page 77, line 23; page 79, line 6-15; in Table 4; throughout the Examples. No new matter has been added.

Reconsideration of the application based on the arguments set forth herein is respectfully requested.

II. Response to the 35 U.S.C. §112, Second Paragraph, Indefiniteness Rejection of Claims 18-23 and 37

Claims 18-23 and 37 have been rejected under 35 U.S.C. § 112, second paragraph, upon the Patent Office contention that the claims are indefinite for failure to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Particularly, the Patent Office asserts that the claims are indefinite because it is not clear whether the method of the claims must be performed on a subject "suffering from sub-optimal urea cycle function" or whether the method may be performed on any "subject in need thereof".

After careful consideration of the rejection and the Patent Office's basis therefore, applicants respectfully traverse the rejection and submit the following remarks.

Without conceding to the assertions of the Patent Office, applicants respectfully submit that independent claims 18, 20 and 37 have been cancelled herein. Furthermore, claims 19 and 21-23 have been amended to depend from claims 35 or 36. No new matter has been added. As such, the instant rejection is believed to be rendered moot.

Accordingly, applicants respectfully request that the instant rejection of claims 19 and 21-23 under 35 U.S.C. § 112, second paragraph, be withdrawn at this time. A Notice of Allowance is also respectfully requested.

III. Response to the 35 U.S.C. § 102(b) Rejection of Claims 1-4, 6-10, 18-23, 25-29 and 36-38 Based on Waugh

Claims 1-4, 6-10, 18-23, 25-29 and 36-38 presently stand rejected under the provisions of 35 U.S.C. § 102(b) as allegedly being anticipated by Waugh. The Patent Office asserts that Waugh teaches each and every element of the rejected claims such that the claims are anticipated.

After careful consideration of the rejection and the Patent Office's basis therefore, applicants respectfully traverse the rejection and submit the following remarks.

Initially, applicants respectfully submit that claims 3, 7, 18, 20, 25, 27 and 37 have been cancelled herein without prejudice. As such, applicants respectfully submit that the instant rejection has been rendered moot with respect to these claims.

Further, applicants respectfully submit that claims 1, 8, 9, 21, 22, 28 and 29 have been amended to recite administration of citrulline. Support for these amendments can be found throughout the specification as originally filed and particularly in original claims 7, 20 and 27. No new matter has been added.

Additionally, applicants respectfully submit that claim 1 has been amended to recite, *inter alia*, "providing a subject under conditions of sub-optimal urea cycle function, wherein the sub-optimal urea cycle function further comprises decreased plasma citrulline". Claim 38 has been amended in a similar manner. Claim 4 has also been amended to recite "decreased plasma citrulline". Support for these amendments can be found throughout the specification as originally filed, and particularly at page 46, lines 13-14; page 49, lines 22-24; page 73, lines 23-31; page 74, lines 10-23; page 76, line 21, through page 77, line 23; page 79, line 6-15; page 91, lines 5-9 and 23-26; in Table 4; throughout the Examples. No new matter has been added.

As such, independent claims 1, 36 and 38 are directed to methods of treatment or prevention in a class of subjects under conditions resulting in decreased plasma citrulline. Applicants respectfully submit that Waugh fails to disclose methods of treatment or prevention directed to such a class of subjects. In particular, Waugh fails to teach a method comprising providing a subject under conditions of sub-optimal urea

cycle function, wherein the sub-optimal urea cycle function further comprises decreased plasma citrulline, as recited in claims 1 and 38. Further, applicants respectfully submit that Waugh fails to teach a method comprising providing a subject suffering from a disorder associated with decreased plasma citrulline, as recited in claim 36. As such, it is believed that Waugh fails to teach each and every element of the claims and therefore does not support a rejection under 35 U.S.C. § 102.

In the instant Official Action the Patent Office contends that "Waugh clearly teaches the administration of citrulline in dosages encompassed by the claims, and administration to subjects with reduced catalytic activities of urea cycle enzymes in association with disease states..." *See*, page 4 of the Official Action. In support of this contention the Patent Office refers to column 13, lines 6-8 of Waugh. However, applicants respectfully submit that the Patent Office appears to have misinterpreted the referenced portion of Waugh.

In particular, applicants respectfully direct the Patent Office's attention to the entire paragraph of Waugh from which the Patent Office quotes. While the Patent Office quotes lines 6-8 of column 13, the entire paragraph, from column 12, line 66, through column 13, line 14, reads as follows:

> I devise, after L. Pauling, (1968), that there exist considerable human individualities in the concentrations and abilities of the many constitutive nitric oxide synthases and of the cell amidinotranferases of L-arginine to glycine for making creatine. I contemplate that increased oral supplementation with L-citrulline leads to increased blood plasma, interstitial fluid, and cell levels of L-arginine to more fully saturate these enzymes both in normal, healthy persons and in individuals with reduced catalytic activities of these enzymes in disease states. Increased reactant levels induced uniquely by L-citrulline supplemention is devised to cause the enzyme reactions to take place at more normal or superior velocities. It is contemplated that better preservation of good health and better treatment of many altered states will result with application of this method of orthomolecular medicine.

(emphasis added).

The Patent Office contends that the "reduced catalytic activities of these enzymes" in the above-referenced portion of Waugh refers to urea cycle enzymes. However, applicants respectfully submit that "these enzymes" refers to nitric oxide synthases and cell amidinotranferases from the preceding sentence, i.e. lines 1-2 of column 13. As would be appreciated by one of ordinary skill in the art, nitric oxide synthases and cell amidinotranferases are not urea cycle enzymes. Rather, as known to those of ordinary skill in the art and according to Nelson and Cox (*Lehninger Principles of Biochemistry, Third Edition*, 2000, Nelson and Cox, Worth Publishers, New York, NY; **Exhibit A**) and J.G. Salway (*Metabolism at a Glance, Second Edition*, 1999, J.G. Salway, Blackwell Publishing, Williston, VT; **Exhibit B**), for example, the urea cycle enzymes include carbamoyl phosphate synthetase I (CPSI), ornithine transcarbamoylase (OTC), argininosuccinate synthetase, argininosuccinate lyase and arginase. Indeed, Waugh makes no mention of CPSI, OTC, argininosuccinate synthetase or argininosuccinate lyase, and only mentions arginase in the context of a quantitative assay for arginine (*See*, column 13, line 33). As such, in contrast to the contentions of the Patent Office, applicants respectfully submit that Waugh does not teach a method comprising administration of citrulline to subjects with reduced catalytic activities of urea cycle enzymes.

Furthermore, Waugh explicitly states that the disclosed supplementation regimes are designed for better health and amelioration of diseases that are not urea-cycle enzyme/substrate liver disorders. *See*, for example, column 10, lines 41-45, of Waugh. As such, applicants respectfully submit that Waugh does not teach supplementation of citrulline to subjects suffering from sub-optimal urea cycle function, comprising decreased plasma citrulline. In marked contrast, Waugh explicitly excludes such classes of subjects and teaches away from the supplementation of citrulline to subjects suffering from sub-optimal urea cycle function. Therefore, when viewed in its entirety, Waugh does not teach administration to subjects with reduced catalytic activities of urea cycle enzymes.

It is well settled that for a cited reference to qualify as prior art under 35 U.S.C. §102, each element of the claimed invention must be disclosed within the reference. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). *See also* M.P.E.P. § 2131. Applicants respectfully submit that Waugh does not support a rejection any of claims 1, 36 and 38 because each and every element as set forth in the claims is not found, either expressly or inherently described, in Waugh. Waugh fails to teach a method comprising providing a subject under conditions of sub-optimal urea cycle function, wherein the sub-optimal urea cycle function further comprises decreased plasma citrulline, as recited in claims 1 and 38. Further, Waugh fails to teach a method comprising providing a subject suffering from a disorder associated with decreased plasma citrulline, as recited in claim 36.

As such, applicants respectfully submit that independent claims 1, 36 and 38 have been distinguished over Waugh. Applicants further submit that claims 2, 4, 6, 8-10, 19, 21-23, 26, 28 and 29 ultimately depend from independent claims 1, 36 and 38. As such, applicants respectfully submit that the rejection of these claims has been addressed as well. Accordingly, applicants respectfully request that the instant rejection of claims 1, 2, 4, 6, 8-10, 19, 21-23, 26, 28, 29, 36 and 38 under 35 U.S.C. § 102(b) be withdrawn at this time. A Notice of Allowance is also respectfully requested.

IV. Response to the 35 U.S.C. § 103(a) Rejection of Claims 5 and 34-35 Based on Waugh and Kaesemeyer

Claims 5 and 34-35 presently stand rejected under the provisions of 35 U.S.C. § 103(a) as allegedly being unpatentable over Waugh in view of Kaesemeyer. Particularly, the Patent Office asserts that Waugh teaches each and every element of the rejected claims, except for the administration of citrulline therapy to a subject suffering from pulmonary hypertension (claim 5) or a subject exposed to or about to be exposed to the environmental stimulus of increased postoperative pulmonary vascular tone associated with cardiac surgery (claim 34). However, the Patent Office asserts that Kaesemeyer makes up for the cited deficiencies of Waugh.

After careful consideration of the rejection and the Patent Office's basis therefore, applicants respectfully traverse the rejection and submit the following remarks.

Initially, applicants respectfully refer to the discussion hereinabove regarding claim 1. In particular, applicants respectfully submit that Waugh does not teach methods wherein the subject is suffering from sub-optimal urea cycle function, wherein the sub-optimal urea cycle function further comprises decreased plasma citrulline, as recited in claim 1. Applicants submit that Kaesemeyer does not cure this deficiency. Kaesemeyer does not appear to teach or suggest treating subjects suffering from sub-optimal urea cycle function, or decreased plasma citrulline.

Thus, the proposed combination of Waugh and Kaesemeyer fails to teach each and every element of claim 1. Given that claims 5 and 34 ultimately depend from claim 1, they are believed to be patentable over the proposed combination of Waugh and Kaesemeyer.

Claim 35 also recites a method comprising providing a subject suffering from a disorder associated with decreased plasma citrulline. Therefore, for at least the same reasons discussed above, claim 35 is also believed to be patentable over the proposed combination of Waugh and Kaesemeyer.

Further, applicants respectfully submit that one of ordinary skill in the art would not have been motivated to combine the references as proposed by the Patent Office. Waugh is directed to orthomolecular medicine which is defined as the treatment of disease by varying the concentrations in the body of substances that are normally present in the body (*see*, e.g., the Title; the Abstract; and column 1, lines 19-24, of Waugh). Kaesemeyer teaches the co-administration of L-arginine (or citrulline) and nitroglycerin (*see*, e.g., the Abstract and columns 5-9 of Kaesemeyer). Nitroglycerin is not normally present in the body. Thus, Kaesemeyer teaches away from the use of substances normally present in the body, as taught by Waugh. As such, one of ordinary skill in the art would not have been motivated to combine Waugh and Kaesemeyer. Rather, one of ordinary skill in the art would have been dissuaded from combining the

orthomolecular medicine of Waugh with the co-administration of non-natural substances, i.e. nitroglycerin, of Kaesemeyer.

Taken together, applicants respectfully submit that the instant 35 U.S.C. §103(a) rejection of claims 5 and 34-35 as allegedly being unpatentable over Waugh in view of Kaesemeyer has been addressed. Accordingly, applicants respectfully request that the rejection of claims 5 and 34-35 be withdrawn at this time. A Notice of Allowance directed to these claims is also respectfully requested.

V. Response to Obviousness Type Double Patenting Rejections

Claims 1-4, 7-10, 18-23, 25-29 and 37-38 have been provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-5, 8-14 and 17-21 of the '117 application. The Examiner contends that although the conflicting claims are not identical, they are not patentably distinct from each other because they are drawn to similar methods and compositions. The Examiner also notes that a non-statutory obviousness-type double patenting rejection can be overcome by filing a terminal disclaimer in compliance with 37 C.F.R. 1.321(c).

Applicants submit herewith a terminal disclaimer in compliance with 37 C.F.R. 1.321(c). In view of the terminal disclaimer, applicants respectfully request withdrawal of the non-statutory obviousness-type double patenting rejection of claims 1-4, 7-10, 18-23, 25-29 and 37-38. Applicants further submit that these claims are in condition for allowance and respectfully solicit the same.

In submitting the attached Terminal Disclaimer, applicants do not acknowledge that the subject matter recited in the conflicting claims are not patentably distinct. Moreover, applicants do not acknowledge that the subject matter recited in the rejected claims of the present patent application is an obvious variation of the subject matter recited in one or more claims in the cited U.S. patents. Indeed, the Federal Circuit has noted that a Terminal Disclaimer "is not an admission of obviousness of the later filed claimed invention in light of the earlier filed disclosure for that is not the basis of the Disclaimer." Quad Environmental Technologies v. Union Sanitary District, 20 U.S.P.Q.2d 1392, 1394 (Fed. Cir. 1991).

The Federal Circuit further noted:

> In legal principle, the filing of a Terminal Disclaimer simply serves the statutory function of removing the rejection of double patenting and raises neither presumption nor estoppel on the merits of the rejection. It is improper to convert this simple expedient "obviation" into an admission or acquiescence or estoppel on the merit.

Quad Environmental Technologies, 20 U.S.P.Q.2d at 1394-95.

Therefore, with the submission of the Terminal Disclaimer provided herewith, applicants are simply availing themselves of the statutory function of removing the double patenting rejection.

VII. Discussion of New Claims 39-42

New claims 39-42 have been added. Support for new claims 39-42 can be found throughout the specification as filed, including particularly at page 46, lines 13-14; page 49, lines 22-24; page 73, lines 23-31; page 74, lines 10-23; page 76, line 21, through page 77, line 23; page 79, line 6-15; in Table 4; throughout the Examples. No new matter has been added.

Applicants respectfully submit that new claims 39-42 are patentable over the references cited by the Patent Office at least for the reasons set forth herein above with respect to independent claims 1, 35, 36 and 38. Applicants further respectfully submit that new claims 39-42 are allowable over the cited art of record. None of the cited art, either alone or in combination, teaches or suggests each and every element of new claims 39-42. Accordingly, allowance of these claims is respectfully requested.

CONCLUSION

Should there be any minor issues outstanding in this matter, the Examiner is respectfully requested to telephone the undersigned attorney. Early passage of the subject application to issue is earnestly solicited.

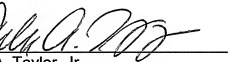
DEPOSIT ACCOUNT

The Commissioner is hereby authorized to charge any fees associated with the filing of this correspondence to Deposit Account Number **50-0426**.

Respectfully submitted,

JENKINS, WILSON, TAYLOR & HUNT, P.A.

Date: 01/26/2009

By: 

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Nitrogen Excretion and the Urea Cycle

If not reused for the synthesis of new amino acids or other nitrogenous products, amino groups are channeled into a single excretory end product (Fig. 18–9). Most aquatic species, such as the bony fishes, excrete amino nitrogen as ammonia and are thus called **ammonotelic** animals; most terrestrial animals are **ureotelic,** excreting amino nitrogen in the form of urea; birds and reptiles are **uricotelic,** excreting amino nitrogen as uric acid. (The pathway for synthesis of uric acid is described in Figure 22–43.) Plants recycle virtually all amino groups, and nitrogen excretion occurs only under very unusual circumstances.

In ureotelic organisms, the ammonia deposited in the mitochondria of hepatocytes is converted to urea in the **urea cycle.** This pathway was

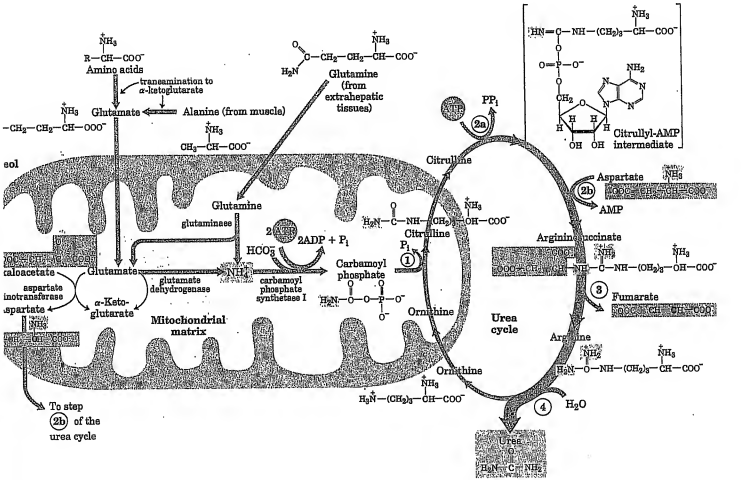

figure 18–9

Urea cycle and reactions that feed amino groups into the cycle. The enzymes catalyzing these reactions (named in the text) are distributed between the mitochondrial matrix and the cytosol. One amino group enters the urea cycle as carbamoyl phosphate (step ①), formed in the matrix; the other (entering at step ②b) enters as aspartate, formed in the matrix by transamination of oxaloacetate and glutamate catalyzed by aspartate aminotransferase. The urea cycle itself consists of four steps: ① Formation of citrulline from ornithine and carbamoyl phosphate; the citrulline passes into the cytosol. ② Formation of argininosuccinate through a citrullyl-AMP intermediate. ③ Formation of arginine from argininosuccinate; this reaction releases fumarate, which enters the mitochondrial citric acid cycle. ④ Formation of urea. The arginase reaction also regenerates the starting compound, ornithine. The pathways by which NH_4^+ arrives in the mitochondrial matrix of hepatocytes are discussed earlier in the chapter.

discovered in 1932 by Hans Krebs (who later also discovered the citric acid cycle) and a medical student associate, Kurt Henseleit. Urea production occurs almost exclusively in the liver and is the fate of most of the ammonia channeled there. The urea passes into the bloodstream and thus to the kidneys and is excreted into the urine. The production of urea now becomes the focus of our discussion.

Urea Is Produced from Ammonia in Five Enzymatic Steps

The urea cycle begins inside liver mitochondria, but three of the subsequent steps occur in the cytosol; the cycle thus spans two cellular compartments (Fig. 18–9). The first amino group to enter the urea cycle is derived from ammonia in the mitochondrial matrix, arising by the multiple pathways described above. The liver also receives some ammonia via the portal vein from the intestine, where it is produced by bacterial oxidation of amino acids. Whatever its source, the NH_4^+ generated in liver mitochondria is immediately used, together with CO_2 (as HCO_3^-) produced by mitochondrial respiration, to form carbamoyl phosphate in the matrix (Fig. 18–10; see also Fig. 18–9). This ATP-dependent reaction is catalyzed by **carbamoyl phosphate synthetase I,** a regulatory enzyme (see below). The mitochondrial form of the enzyme is distinct from the cytosolic (II) form, which has a separate function in pyrimidine biosynthesis (Chapter 22).

The carbamoyl phosphate, which may be regarded as an activated carbamoyl group donor, now enters the urea cycle. The cycle has four enzymatic steps. First, carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline, with the release of P_i (Fig. 18–9, step ①). Ornithine thus plays a role resembling that of oxaloacetate in the citric acid cycle, accepting material at each turn of the cycle. The reaction is catalyzed by **ornithine transcarbamoylase,** and the citrulline that results passes from the mitochondrion to the cytosol.

The second amino group is introduced from aspartate (generated in mitochondria by transamination and transported into the cytosol) by a condensation reaction between the amino group of aspartate and the ureido (carbonyl) group of citrulline, forming argininosuccinate (step ②). This cytosolic reaction, catalyzed by **argininosuccinate synthetase,** requires ATP and proceeds through a citrullyl-AMP intermediate. The argininosuccinate is then reversibly cleaved by **argininosuccinate lyase** (step ③) to form free arginine and fumarate, the latter entering mitochondria to join the pool of citric acid cycle intermediates. In the last reaction of the urea cycle (step ④), the cytosolic enzyme **arginase** cleaves arginine to yield **urea** and ornithine. Ornithine is transported into the mitochondrion to initiate another round of the urea cycle.

As we noted in Chapter 15, the enzymes of many metabolic pathways are clustered (p. 541), the product of one enzyme reaction being channeled directly to the next enzyme in the pathway. In the urea cycle, the mitochondrial and cytosolic enzymes appear to be clustered in this way. The citrulline transported out of the mitochondrion is not diluted into the general pool of metabolites in the cytosol but is passed directly to the active site of argininosuccinate synthetase. This channeling between enzymes continues for argininosuccinate, arginine, and ornithine. Only urea is released into the general cytosolic pool of metabolites.

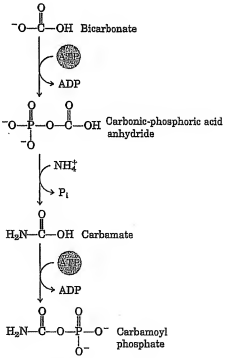

figure 18–10
Reaction catalyzed by carbamoyl phosphate synthetase I. The terminal phosphate groups of two molecules of ATP are used to form one molecule of carbamoyl phosphate. In other words, this reaction has two activation steps.

Exhibit B

The ornithine cycle for the production of urea: 'the urea cycle'

16

Chart 16.1 (opposite) Nitrogen, in the form of ammonium ions or glutamate, is used for urea synthesis.

A study of the other metabolic cycle elucidated by Krebs, the 'Krebs-Henseleit ornithine cycle' popularly (but inaccurately) known as the 'urea cycle', offers an overview of amino acid metabolism. In the fed state, any amino acids surplus to requirement for protein synthesis can be metabolized to non-nitrogenous substances such as glucose, glycogen or fatty acids, or they can be oxidized to generate ATP. On the other hand, during fasting or starvation, catabolic wasting of muscle occurs thereby yielding amino acids which are used for gluconeogenesis so as to maintain normoglycaemia. Because the ammonia derived from these amino acids is extremely toxic, it is converted to non-toxic urea for urinary excretion. Any ammonia which evades detoxification as urea can alternatively be incorporated into glutamine by glutamine synthetase, which has been described by Hitunziger as serving as a scavenger for stray ammonium ions.

The origins of the nitrogen used for urea synthesis

In the fed state, amino acids are formed from dietary proteins by proteolytic digestion in the gastrointestinal tract. The amino acids are then absorbed into the bloodstream and may be used intact for protein synthesis. Alternatively, surplus amino acids can be metabolized to glucose, be used for fatty acid synthesis, or be catabolized to generate ATP. The amino groups are removed by transamination and deamination prior to urea synthesis in the peripheral hepatocytes. The residual carbon skeletons are metabolized to the gluconeogenic precursors: pyruvate, succinyl CoA, fumarate, α-ketoglutarate, oxaloacetate or, alternatively, to the ketone bodies or their precursors (see Chapters 30 and 35 respectively).

In starvation, the circulating amino acids are derived mainly from proteolysis of muscle protein. Transamination of the amino acids, particularly the branched-chain amino acids isoleucine, valine and leucine (see Chart 16.1) occurs in the muscle in partnership with pyruvate, so that the amino acid pool in the venous blood draining from the muscle is enriched with alanine (see Chapter 18). This alanine is transported to the liver, entering via the hepatic artery, where transamination with α-ketoglutarate (α-KG) occurs to form glutamate.

Chart 16.1: Nitrogen, in the form of ammonium ions or glutamate, is used for urea synthesis

As shown in Chart 16.1, amino acids, whether of dietary or endogenous (muscle) origin, enter the pathway for urea synthesis by the transdeamination route or the transamination route.

Transdeamination route

This route consists of an initial transamination in the cytosol, followed by deamination in the mitochondrion. Initially α-ketoglutarate accepts an amino group from the donor amino acid to form glutamate in a cytosolic reaction catalysed by an aminotransferase. The glutamate is then transported by the glutamate carrier into the mitochondrion where it is oxidatively deaminated by glutamate dehydrogenase to form α-ketoglutarate and ammonium ions. The ammonium is incorporated into carbamoyl phosphate, which in turn reacts with ornithine to enter the urea cycle as citrulline.

Transamination route

Alternatively, nitrogen from the amino acids can enter the urea cycle via the transamination route, which involves two transamination reactions. Again, α-ketoglutarate initially accepts the amino group from the donor amino acid and once again glutamate is formed as described above. However, a second transamination now follows, with oxaloacetate accepting the amino group from glutamate to form aspartate in a reaction catalysed by aspartate aminotransferase (AST). This aspartate now carries the second amino group into the urea cycle by condensing with citrulline to form argininosuccinate. Argininosuccinate is then cleaved to form fumarate and arginine. Finally, arginine is hydrolysed to ornithine and urea, and ornithine is regenerated for another rotation of the cycle.

Regulation of the urea cycle

The condensation of ammonia with bicarbonate to form carbamoyl phosphate is catalysed by carbamoyl phosphate synthetase (CPS) which is only active in the presence of its allosteric effector, *N*-acetylglutamate (NAG). NAG is synthesized from acetyl CoA and glutamate by *N*-acetylglutamate synthase.

Disorders of the urea cycle

The most common urea cycle disorder is ornithine transcarbamoylase (OTC) deficiency which is X-linked. Affected boys develop severe hyperammonaemia which often leads to early death. However, in heterozygous girls, the condition can vary from being undetectable to the severely seen in boys. In this condition, carbamoyl phosphate accumulates and passes into the cytosol where it reacts with aspartate to form carbamoyl aspartate. This

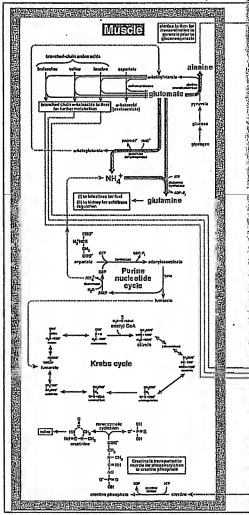

Exhibit B

is metabolized to form orotate by the reactions described for pyrimidine synthesis in Chapter 34. Detection of orotic acid in urine is used to diagnose OTC deficiency.

Creatine and creatinine

The main function of the ornithine cycle is to produce urea. However, as shown in the chart, a small but significant quantity of arginine is diverted to form creatine. This is phosphorylated by creatine kinase to produce creatine phosphate which is the phosphagen used to generate ATP during short bursts of intensive exercise. Approximately 2% of the body pool of creatine phosphate spontaneously cyclizes each day and is excreted in the urine as creatinine.

The purine nucleotide cycle

The purine nucleotide cycle described by Lowenstein, although present in many types of tissues, is particularly active in muscle. During vigorous exercise in rats, the blood concentration of ammonium ions can increase five-fold. This ammonium is thought to be derived from aspartate via the purine nucleotide cycle. This cycle is mentioned again in Chapter 31.